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Associations between a fatty acid desaturase gene polymorphism and blood arachidonic acid compositions in Japanese elderly

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ABSTRACT

We investigated whether the single nucleotide polymorphism rs174547 (T/C) of the fatty acid desaturase-1 gene, *FADS1*, is associated with changes in erythrocyte membrane and plasma phospholipid (PL) long-chain polyunsaturated fatty acid (LCPUFA) composition in elderly Japanese participants ($n=124$; 65 years or older; self-feeding and oral intake). The rs174547 C-allele carriers had significantly lower arachidonic acid (ARA; *n*-6 PUFA) and higher linoleic acid (LA, *n*-6 PUFA precursor) levels in erythrocyte membrane and plasma PL (15% and 6% ARA reduction, respectively, per C-allele), suggesting a low LA to ARA conversion rate in erythrocyte membrane and plasma PL of C-allele carriers. α-linolenic acid (*n*-3 PUFA precursor) levels were higher in the plasma PL of C-allele carriers, whereas levels of the *n*-3 LCPUFAs eicosapentaenoic acid (EPA) or docosahexaenoic acid (DHA) were unchanged in erythrocyte membrane and plasma PL. Thus, rs174547 genotypes were significantly associated with different ARA compositions of the blood of elderly Japanese.

1. Introduction

Arachidonic acid (ARA), eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA), constituents of biological membranes and nervous tissue, have multiple activities including modulation of inflammatory responses through the formation of eicosanoids and regulatory effects on transcription factors [1]. Long-chain polyunsaturated fatty acids (LCPUFA) such as ARA and DHA are sourced both from the diet and endogenously from desaturation and elongation of their dietary precursors, linoleic acid (LA), an *n*-6 PUFA (polyunsaturated fatty acids), and α-linolenic acid (ALA), an *n*-3 PUFA, respectively. These precursors are metabolized by the endogenous desaturase enzymes ⊿6 desaturase and ⊿5 desaturase. ⊿6 desaturase catalyzes the conversion of LA and ALA to γ-linolenic acid (GLA) and stearidonic acid, respectively, while ⊿5 desaturase catalyzes the conversion of dihomo-γ-linolenic acid (DGLA) and eicosatetraenoic acid (ETA) to ARA and EPA, respectively. The activity of ⊿6 desaturase and ⊿5 desaturase is involved in regulation of the levels of pro-inflammatory and anti-inflammatory eicosanoids that are generated from PUFA [2] and has been reported to be associated with the development of allergies [3], obesity [4], and cardiovascular diseases [5].

In recent years, single nucleotide polymorphisms (SNPs) of each of the ⊿6 desaturase and ⊿5 desaturase genes, fatty acid desaturase *FADS2* and *FADS1*, respectively, have been shown to be associated with differences in the expression [8] or the activity of the enzyme, and to be associated with the concentration of LCPUFA in plasma and red blood cells by genome-wide association analyses [3,6,7,9–11]. However, in a report that compared the fatty acids desaturase (*FADS*) rs174537 genotype of 166 European-Americans with that of 63 African-Americans [11], the serum ARA composition in European minor allele carriers was lower than that of the major allele carriers, but there was no difference in serum ARA composition between major and minor African-American allele carriers; therefore ethnic differences in the effect of the rs174537 genotype were observed.

Abbreviations: ALA, α-linolenic acid; ARA, arachidonic acid; BMI, body mass index; DGLA, dihomo-γ-linolenic acid; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; ETA, eicosatetraenoic acid; FADS, fatty acid desaturase; GLA, γ-linolenic acid; LA, linoleic acid; LCPUFA, long chain polyunsaturated fatty acid; PL, phospholipid; SNP, single nucleotide polymorphism

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In addition, in a study that compared the *FADS* rs174547 genotype of Caucasians and Asians [12], the C allele was found to be a minor allele in Caucasians, whereas it was a major allele in Asians; thus, the frequency of occurrence of these alleles differs by ethnic group. Therefore, even alleles of the same SNP can be present at different frequencies and can be differently associated with blood ARA levels depending on ethnicity.

Schaeffer et al. have shown that there is a relationship between the SNPs in *FADS2* and *FADS1* genes and the fatty acid composition, and in particular, the ARA composition of serum phospholipids; these SNPs could explain the 28% variance of ARA [3]. In our previous cross-sectional study of blood phospholipid ARA composition, a relationship between blood phospholipid ARA composition and LA or ARA intake was not observed; instead, ARA levels were shown to be inversely related to EPA and DHA intake [13]. ARA, EPA, and DHA are all LCPUFA constituents of membrane phospholipids. If dietary EPA and DHA intake is large, then incorporation of ARA into phospholipids is considered to be relatively reduced. Therefore, considering the high intake of fish by Japanese people and the importance of ethnicity for *n*-6 PUFA and *n*-3 PUFA levels, it is necessary to consider whether *FADS* gene polymorphism is associated with the metabolism of *n*-6 and *n*-3 LCPUFA in the Japanese population.

Since it has been reported that the genotype of rs174547 is associated with differences in the lipid metabolism of Japanese people [14], in the present study, we evaluated the impact of rs174547 on erythrocyte membrane and plasma PL fatty acids. Because inter-individual variability of EPA and DHA intake of Japanese people is very large, it is expected that its impact on blood fatty acid composition will be large. Therefore, in the present study, we specifically focused on an elderly Japanese population in whom EPA and DHA intake was fixed long-term by being on a managed diet for more than six months.

2. Methods

2.1. Study participants

The study subjects comprised 132 subjects, 65 years of age or older, who were able to feed themselves orally, and who were admitted to a hospital in the western part of Saitama Prefecture for more than six months. Exclusion criteria included those receiving a special diet including tube feeding, parenteral nutrition, those unable to consume meals by mouth, those suffering from an acute illness, or those deemed inappropriate to participate at a physician's discretion. Of the eligible candidates, five were unable to give informed consent, two were unable to provide blood samples due to poor health on the collection day, and one needed to change hospitals; therefore, a total of 124 of the 132 candidates participated.

The study was performed after obtaining approval from the medical ethics committee of Kagawa Nutrition University, the Mamie Hospital Ethics Review Committee, the Ethics Committee of Niigata Prefecture University, the Ethics Committee of Jichi Medical University, and the Ethics Committee on Human Experimentation of Suntory Holdings Ltd., which conformed to the principles set forth in the Declaration of Helsinki. All participants or the family received a verbal explanation of the study and provided written informed consent.

2.2. Dietary assessment

For all subjects, dietary intake was recorded continuously for one week prior to blood collection at the hospital. The nurse evaluated meals using a 10-stage intake for each of the meals provided by the hospital, as well as snacks and drinks.

The weights of foods consumed were estimated from the hospital's meal cart. Commercially prepared foods were assigned values based on the raw materials and noted. The Standard Tables of Food Composition 2010 [15] in Japan were used as a reference for determination of intake of energy, protein, fat, and carbohydrates, with the Fifth Revised and Enlarged Edition of the Tables of Fatty Acid Composition [16] in Japan as a reference.

2.3. Blood sampling

Blood sampling was conducted after completing the one week dietary survey. A vacuum sampling tube with EDTA-2Na as an anticoagulant was used for blood collection. The samples were centrifuged at 1600*g* for 10 min to separate plasma and erythrocytes. The plasma and erythrocytes obtained were used to measure FA composition. All samples were stored at −80 °C until analyzed. Venous blood samples were collected to obtain DNA samples. Blood fatty acids were analyzed for erythrocyte membrane and plasma PL fraction [17].

2.4. Fatty acid analysis of blood lipid fractions

The preparation of erythrocyte membranes and analysis of FA composition were conducted as previously described [17]. Briefly, erythrocytes were washed twice with saline to remove the buffy coat. Next, 0.01 M Tris–HCl buffer (pH 7.4) was added to the pellet to hemolyze the erythrocytes. Centrifugation was performed at 10,000*g* for 20 min to obtain only the erythrocyte membranes. The total FAs of the erythrocyte membranes and plasma were extracted by the method of Folch et al. [18]. The FAs of erythrocyte membrane and plasma PL were separated by thin-layer chromatography, and, after transmethylation with HCl–methanol, the FA composition was analyzed using a GL Sciences GC-4000 gas chromatograph (GL Sciences Inc., Tokyo, Japan) with a DB-225 capillary column (J&W Scientific Inc., Folsom, CA, USA). Erythrocyte membranes and plasma fatty acids are presented as a percentage of the total FA composition [17].

2.5. Genotyping

For DNA extraction, an all-automated nucleic acid extraction system (Magtration System 6GC; Precision System Science, Chiba, Japan) was used for whole blood, and then a special reagent (MagDEA DNA 200 (GC); Precision System Science Co., Ltd.; Chiba, Japan) was used. The rs174547 (a T/C polymorphism in intron 9 of *FADS1*) genotypes in all participants were determined using TaqMan genotyping Assay Systems (Applied Biosystems, Foster City, CA) [14].

2.6. Statistical methods

Comparisons among the groups of genotypes were determined using analysis of variance (ANOVA) for data of age and body mass index (BMI). Multiple comparisons were made using Tukey's test ($P < 0.05$), and, for data of categorical variables, were determined using χ^2 analyses for data of categorical variables. The association of the score with clinical history was assessed by means of Fisher's exact test. Comparisons among the fatty acid intake of groups were determined using analysis by the Kruskal–Wallis test. Comparisons among the groups in the case of normal distribution were determined using analysis of covariance (ANCOVA) models, adjusted for sex, age, BMI, and clinical history as a covariate. Multiple comparisons were made using Tukey's test ($P < 0.05$). We based surrogate estimates of desaturase activity on available ratios of *n*-6 PUFA. ARA/DGLA was used to estimate $\Delta 5$ desaturase activity (*FADS1*), ARA/LA in the *n*-6 pathway [5,12]. The ratios of